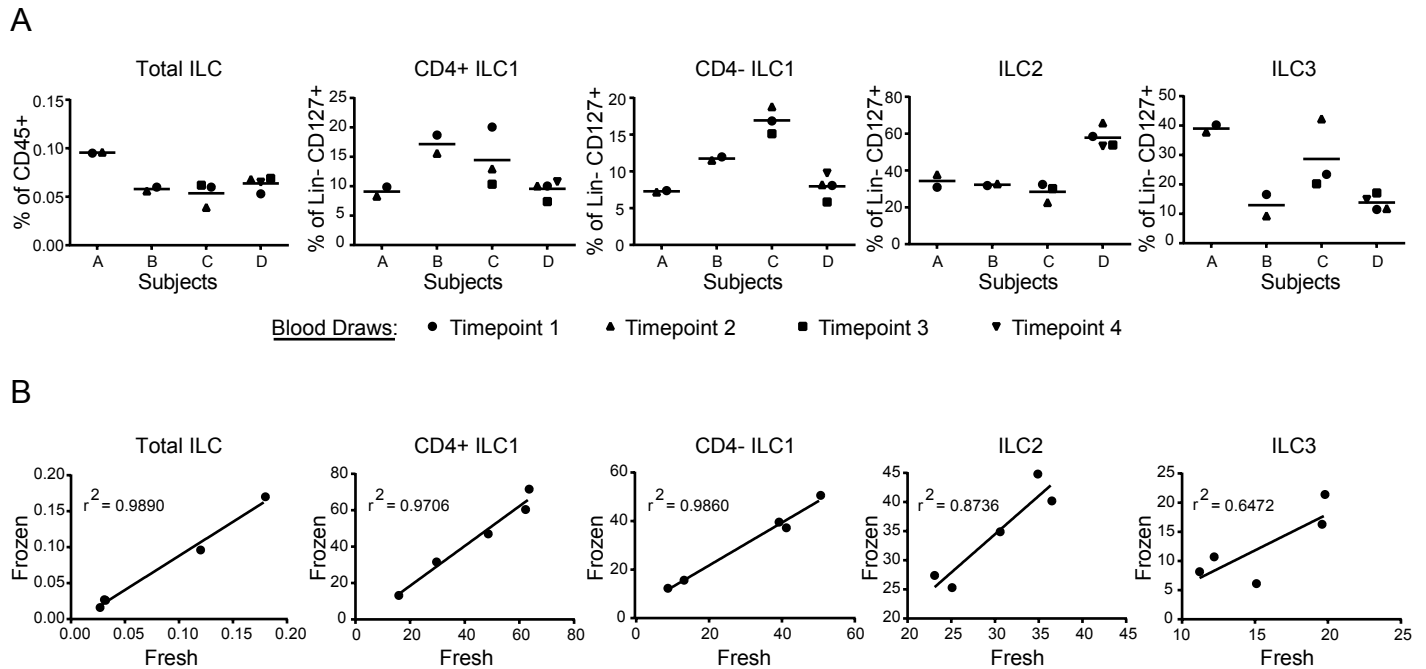
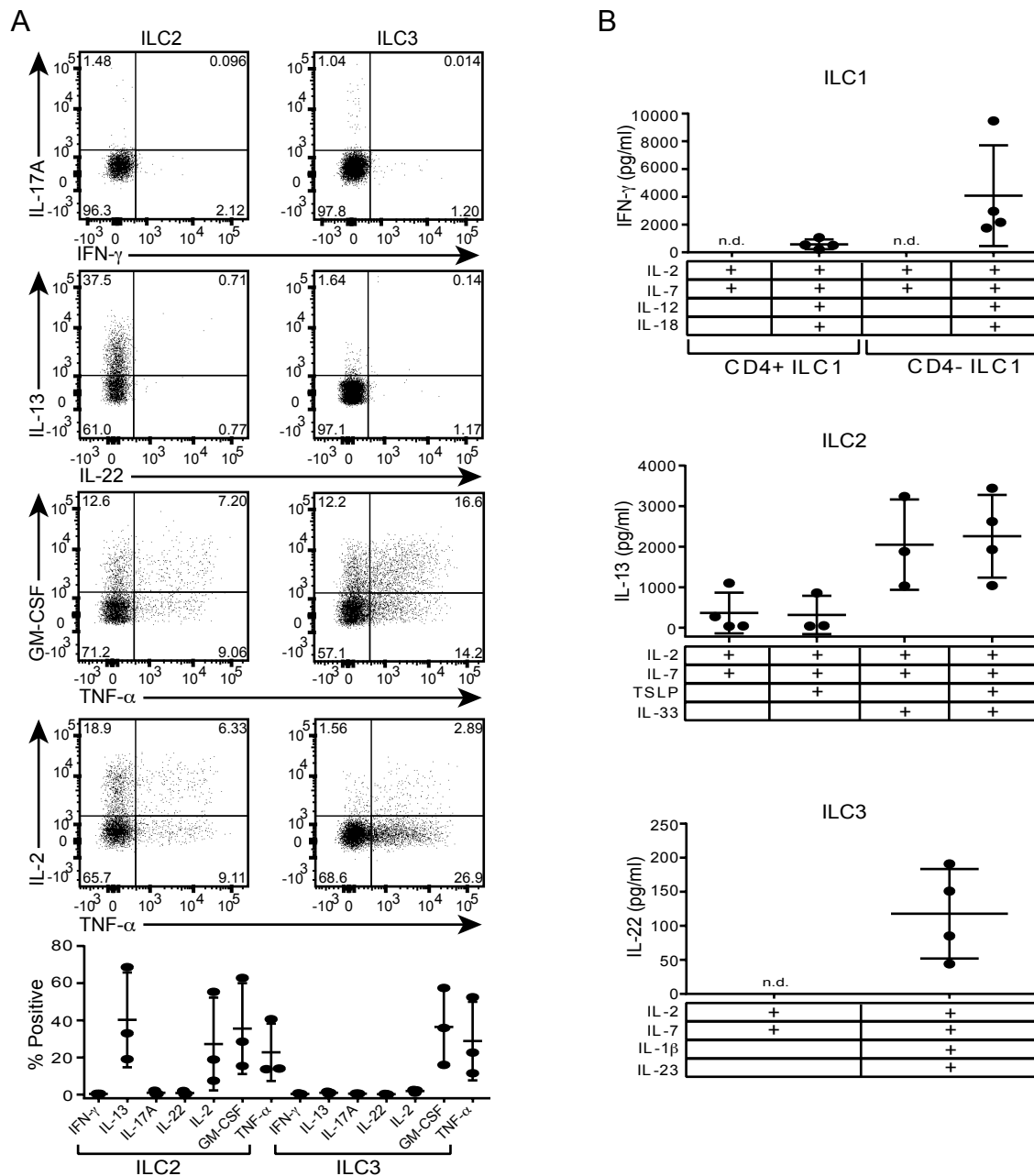


Supplemental Figure 1. CD16, perforin and granzyme expression in ILC subsets.

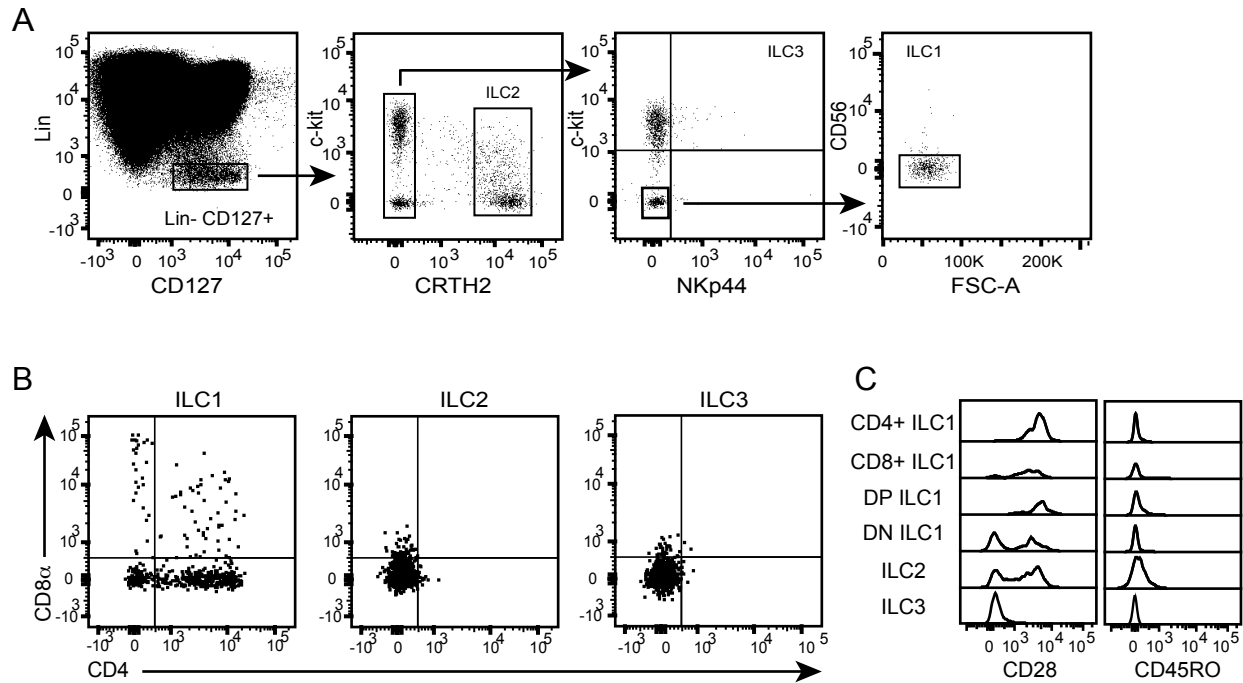
Frozen PBMC were thawed and stained for surface markers (including CD56 and CD16), and intracellular expression of perforin (Perf), Granzyme A (GrzA), and Granzyme B (GrzB) was analyzed in ILC subsets by flow cytometry. CD16 was not included in the lineage in these analyses. CD56 was not gated out in ILC1 to demonstrate the correlation between CD56 expression and CD16, perforin and granzyme expression. Flow plots are from a single representative subject (n = 5).



Supplemental Figure 2. Stability of ILC frequencies with freezing and over time. **(A)** Frozen PBMC from blood drawn at 2-4 different time points from the same individuals (subjects A-D) were thawed and analyzed for ILC subset composition by flow cytometry. Each point for a single subject represents a separate draw. **(B)** Fresh PBMC were isolated from peripheral blood and then analyzed for ILC subsets by flow cytometry. PBMC were frozen concurrently then thawed > 1 week later, and ILC subset frequencies were determined by flow cytometry. Total ILC are expressed as a percentage of live CD45+ lymphocytes, and individual ILC subsets as a percentage of Lin- CD127+ within live CD45+ lymphocytes. Each point represents a blood draw from a single individual (n = 5).



Supplemental Figure 3. Cytokine production by the ILC subsets. Fresh PBMC were lineage-depleted using MACS columns, stained for ILC surface markers, and flow sorted for ILC subsets. **(A)** Sorted ILC2 and ILC3 were stimulated with PMA/ionomycin/brefeldin A for 6 hours and then examined for intracellular cytokine expression by flow cytometry. Flow plots are from a single representative individual ($n = 3$). **(B)** Sorted CD4⁺ ILC1, CD4⁻ ILC1, ILC2 and ILC3 were cultured with the indicated cytokines for 18 hours. Supernatant was collected, and cytokine production was determined by ELISA ($n = 3 - 4$).



Supplemental Figure 4. Cord blood ILC subsets. **(A)** Previously frozen mononuclear cells from cord blood were thawed and stained for ILC1, ILC2 and ILC3. **(B)** CD4 and CD8 α staining in cord blood ILC subsets. **(C)** CD45RO and CD28 expression on cord blood ILC subsets. Flow plots and histograms are from a single representative sample (n = 5).